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SYNTHESIS AND CHARACTERIZATION OF AN OLIGONUCLEOTIDE CONTAINING THE BIFURCATED NUCLEOBASE ω-ADENYLPROPYL URACIL

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Abstract. A novel bifurcated nucleoside that bears an adenine group trimethylene linked to the 5-position of uracil, 5-(omega-adenylpropyl)-2'-deoxyuridine, has been synthesized and incorporated into a DNA dodecamer. This nucleoside is designed to both intercalate and hydrogen bond when incorporated into an oligonucleotide helix. Initial results of its influence on helix properties are reported. Copyright © 1996 Elsevier Science Ltd

Intercalators distort the structures of nucleic acid helices and compensate for this distortion by way of favorable interactions.[1] One means for increasing the stability of intercalated complexes is to reduce or eliminate the entropic cost of bimolecular binding. In the past this has been accomplished through bis-intercalators[2] and anchoring an intercalator directly to an oligonucleotide.[3] We seek to examine the effect of incorporating a group into an oligonucleotide that is at once capable of both hydrogen bonding to an opposite strand base and intercalation. Towards this end, the synthesis of a uridine derivative bearing a trimethylene linked adenine at the 5-position is reported, along with its incorporation into a DNA dodecamer. The design of this nucleoside is based upon the work of Leonard et al.[4] detailing physical interactions of pyrimidine and purine dimers connected by way of hydrocarbon chains.

Due to synthetic expedience, 2'-deoxyuridylate was chosen to bear the tethered base. Adenine was chosen as the base to be tethered because of its superior ability to π-stack. The trimethylene linker was decided upon because of its tendency to promote optimal stacking when it separates two bases.[4] The synthesis of 5-(ω-adenylpropyl)-2'-deoxyuridine in a form suitable for oligonucleotide synthesis is shown in Figures 1 and 2. N^9-Propynyl adenine was prepared by the method of Joshi and Zemlicka.[5] This material was converted into its N^6-dibenzoyl derivative **3**[6] rather than a monobenzoyl one because when the latter was employed, solubility problems were later encountered. The coupling[7] of dibenzoyl propynyl adenine **3** to 5-iodo-2'-deoxyuridine (**4**) was performed using a slight modification[8] of the method reported by Hobbs.[9] Transformation of nucleoside **6**[10] bearing a trimethylene linker into phosphoramidite **8** was accomplished by standard methods.[11]

The following dodecanucleotide containing a single 5-(ω-adenylpropyl)-2'-deoxyuridylate was synthesized: 5'-d-AAAAAAUAAAAA-3' (**9**). After automated synthesis, deprotection of the oligomer was performed using standard conditions of concentrated ammonium hydroxide at 55 °C for 15 hours. Purification of **9** was effected by reverse-phase HPLC. Incorporation of the intact modified base was shown by MALDI-TOF mass spectrometry, where the correct parent ion was observed (Figure 3). Purity of the oligomer was assessed as greater than 95% based on end-labelling followed by PAGE. An examination of the duplex between oligomer **9** and 5'-d-T_5AT_6 gave a T_m = 33 °C which was essentially identical (within 1 °C) to that of the all natural duplex counterpart having thymine in place of the bifurcated base.

In an appropriate context, oligonucleotides bearing bifurcated bases could find use in the stabilization of nucleic acid complexes through intercalation and/or hydrogen-bonding. Alternatively, tethered extrahelical bases might enable novel supermolecular interactions.

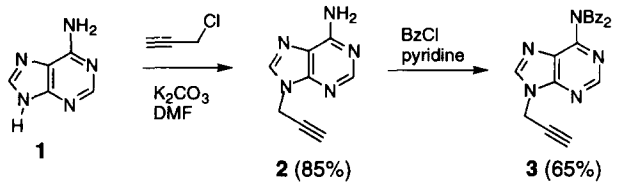

Figure 1.

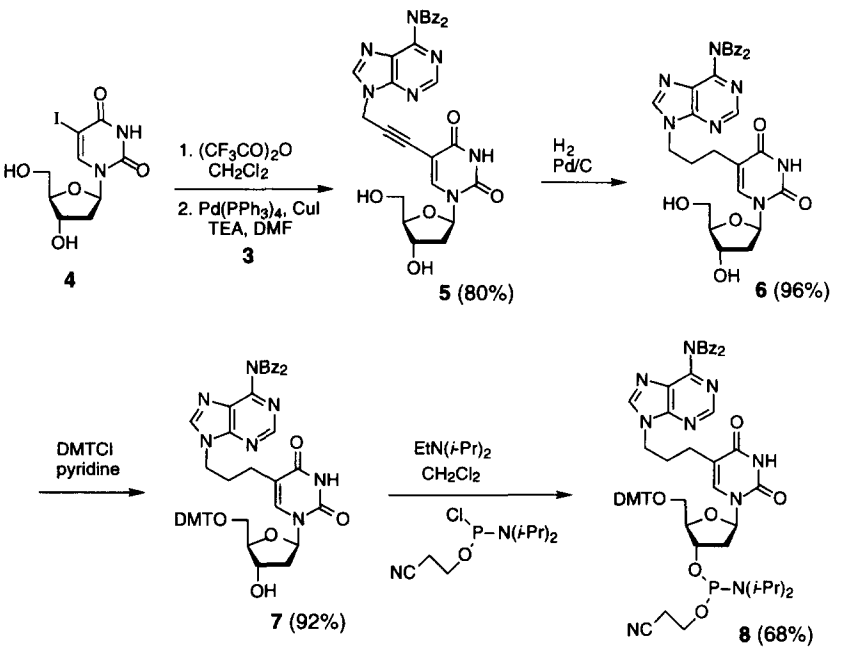

Figure 2.

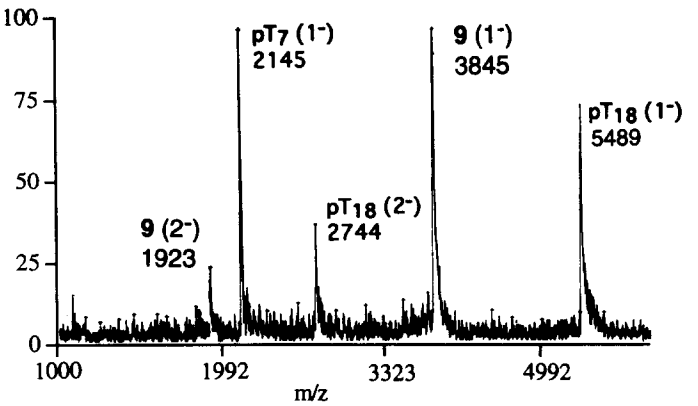

Figure 3. Laser desorption mass spectrum of 5'-d-AAAAAAUAAAAA-3' (**9**); U = 5-(ω-adenylpropyl)-2'-deoxyuridylate. Calculated masses ([M-H]$^-$) are (**9**): 3848; pT_7: 2146; and pT_{18}: 5493.

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References.

1. Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

2. (a) Dervan, P. B.; Becker, M. M. *J. Am. Chem. Soc.* **1978**, *100*, 1968; (b) Veal, J. M.; Li, Y.; Zimmerman, S. C.; Lamberson, C. R.; Cory, M.; Zon, G.; Wilson, W. D. *Biochemistry* **1990**, *29*, 10918; (c) Spielmann, H. P.; Wemmer, D. E.; Jacobsen, J. P. *Biochemistry* **1995**, *34*, 8542.

3. (a) Letsinger, R. L.; Schott, M. E. *J. Am. Chem. Soc.* **1981**, *103*, 7394; (b) Jäger, A.; Levy, M. J.; Hecht, S. M. *Biochemistry* **1988**, *27*, 7237; (c) Thuong, N. T.; Chassignol, M. *Tetrahedron Lett.* **1988**, *29*, 5905; (d) Grigoriev, M.; Praseuth, D.; Robin, P.; Hemar, A.; Saison-Behmoaras, T.; Dautry-Varsat, A.; Thuong, N. T.; Hélène, C.; Harel-Bellan, A. *J. Biol. Chem.* **1992**, *267*, 3389; (e) Chen, J.-K.; Carlson, D. V.; Weith, H. L.; O'Brien, J. A.; Goldman, M. E.; Cushman, M. *Tetrahedron Lett.* **1992**, *33*, 2275; (f) Mouscadet, J.-F.; Ketterlé, C.; Goulaouic, H.; Carteau, S.; Subra, F.; Le Bret, M.; Auclair, C. *Biochemistry* **1994**, *33*, 4187; (g) Zhou, B.-W.; Puga, E.; Sun, J.-S.; Garestier, T.; Hélène, C. *J. Am. Chem. Soc.* **1995**, *117*, 10425.

4. (a) Leonard, N. J.; Ito, K. *J. Am. Chem. Soc.* **1973**, *95*, 4010; (b) Leonard, N. J. *Acc. Chem. Res.* **1979**, *12*, 425.

5. Joshi, R. V.; Zemlicka, J. *Tetrahedron Lett.* **1993**, *49*, 2353.

6. 6-N,N-Dibenzoyl-9-propargyladenine (**3**). To a solution of 9-Propargyladenine[5] **2** (460 mg, 2.67 mmol) in pyridine at 0°C was added benzoyl chloride (1.5 mL, 10.7 mmol). The mixture was warmed to RT and allowed to stir for 6 h. The pyridine was removed by evaporation, the residue dissolved in CH_2Cl_2, and subsequently washed with 5% aq. $NaHCO_3$. The organic phase was dried over Na_2SO_4 and evaporated to give a residue, which was chromatographed on SiO_2 with 3% MeOH/CH_2Cl_2 to yield 840 mg of **3** (65%): ^{1}H NMR ($CDCl_3$) δ: 2.57 (s, 1 H), 5.04 (s, 2 H), 7.33-7.51 (m, 6 H), 7.86 (d, J = 7.8 Hz, 4 H), 8.28 (s, 1 H), 8.68 (s, 1 H). ^{13}C

NMR ($CDCl_3$) δ: 33.36, 75.30, 75.57, 127.18, 128.67, 129.36, 132.96, 133.97, 144.09, 151.74, 152.25, 152.61, 172.21. HRMS (FAB^+) calcd for $C_{22}H_{16}N_5O_2$ 382.1304 (MH^+), found 382.1293.

7. 5-[ω-(6-N,N-Dibenzoyladen-9-yl)propynyl]-2'-deoxyuridine (**5**). To a suspension of 5-iodo-2'-deoxyuridine (**4**) (708 mg, 2.00 mmol) in CH_2Cl_2 (12 mL) was added trifluoroacetic anhydride (2.54 mL, 18.0 mmol) at room temperature. The mixture was stirred overnight. After concentration of the mixture, the residue was dried in vacuo at room temperature to give 1.09 g of 3',5'-di-O-trifluoroacetyl-5-iodo-2'-deoxyuridine as a solid foam, which was used without further purification in the next step. To this material, along with **3** (2.288 g, 6.00 mmol), tetrakis(triphenylphosphine)palladium (0) (462 mg, 0.40 mmol), and copper (I) iodide (152 mg, 0.80 mmol) were added dry DMF (20 mL) and Et_3N (0.558 mL, 4.00 mmol). The mixture was stirred at RT for 48 h, and then concentrated under a vacuum. The residue was purified by chromatography (SiO_2, 5%-10% MeOH/CH_2Cl_2). The fraction containing **5** was concentrated and treated with anion exchange resin (AG1X8, HCO_3^-, 1.71 g, 3.0 eq.) in 20 mL of 1/1, CH_2Cl_2/MeOH at room temperature for 30 min. Evaporation of the solvent gave **5** (977 mg, 80%): ^1H NMR (DMSO-d_6) δ: 2.15 (m, 2 H), 3.60 (m, 2 H), 3.81 (m, 1 H), 4.24 (m, 1 H), 5.11 (t, J = 5.0 Hz, 1 H), 5.27 (d, J = 4.2 Hz, 1 H), 5.43 (s, 2 H), 6.11 (t, J = 6.5 Hz, 1 H), 7.47 (m, 4 H), 7.60 (m, 2 H), 7.81 (d, J = 7.5 Hz, 4 H), 8.29 (s, 1 H), 8.73 (s, 1 H), 8.74 (s, 1 H), 11.70 (s, 1 H). ^{13}C NMR (DMSO-d_6) δ: 34.03, 60.90, 70.02, 78.11, 84.91, 85.55, 87.65, 97.12, 126.61, 129.04, 133.39, 133.46, 144.68, 146.42, 149.38, 150.81, 151.90, 152.50, 161.52, 172.07. MS (FAB^+) *m/z*: 608 (MH^+). HRMS (FAB^+) calcd for $C_{31}H_{26}N_7O_7$ 608.1894 (MH^+), found 608.1904.

8. Hashimoto, H.; Nelson, M. G.; Switzer, C. *J. Am. Chem. Soc.* **1993**, *115*, 7128.

9. Hobbs, F. W. *J. Org. Chem.* **1989**, *54*, 3420.

10. 5-[ω-(6-N,N-Dibenzoyladen-9-yl)propyl]-2'-deoxyuridine (**6**). To compound **5** (182 mg, 0.30 mmol) was added CH_2Cl_2/MeOH (1/1, 6 mL), and the mixture was heated gently until a solution was obtained. 10% Pd/C (60 mg) was added, and the resulting mixture was stirred under H_2 pressure (50 psi) at RT for 48 h. The mixture was filtered through celite and concentrated. Chromatography (SiO_2, 5%-10% MeOH/CH_2Cl_2) gave **6** (177 mg, 96%) as a white solid: ^1H NMR (DMSO-d_6) δ: 2.0-2.3 (m, 6 H), 3.57 (m, 2 H), 3.76 (m, 1 H), 4.2-4.3 (m, 3 H), 5.03 (t, J = 5.1 Hz, 1 H), 5.24 (d, J = 4.2 Hz, 1 H), 6.17 (t, J = 6.6 Hz, 1 H), 7.46 (m, 4 H), 7.59 (m, 2 H), 7.75 (s, 1 H), 7.77 (d, J = 7.5 Hz, 4 H), 8.63 (s, 1 H), 8.67 (s, 1 H), 11.33 (s, 1 H). ^{13}C NMR (DMSO-d_6) δ: 23.63, 27.92, 43.22, 61.23, 70.33, 83.99, 87.31, 112.19, 126.79, 128.77, 128.99, 133.31, 133.55, 136.71, 147.20, 150.33, 150.61, 151.53, 153.07, 163.35, 172.07. MS (FAB^+) *m/z*: 612 (MH^+). HRMS (FAB^+) calcd for $C_{31}H_{30}N_7O_7$ 612.2207 (MH^+), found 612.2227.

11. Atkinson, T.; Smith, M. In *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J. Ed.; IRL: Oxford, 1985.

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